Psittacine beak and feather disease in a free-living ring-necked parakeet (Psittacula krameri) in Great Britain

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Abstract: The ring-necked parakeet (RNP), (Psittacula krameri), is an invasive species in Great Britain (GB) which is undergoing rapid population expansion in the wild. Although it has been suggested that RNPs could be a potential source of infectious disease, little research has been done on the pathogens infecting this species in GB. Psittacine beak and feather disease (PBFD), caused by beak and feather disease virus (BFDV), is an important infectious disease of psittacines, including captive RNPs, in GB and elsewhere. A wild RNP with marked feather abnormalities observed in an urban garden in London was euthanased and examined post mortem. Plucked contour feathers and pooled liver and spleen were PCR positive for BFDV DNA. Histopathological examination of affected skin demonstrated BFDV-compatible lesions. A feather from another RNP from a different location also was PCR-positive for BFDV. This is the first report of PBFD in a wild free living bird in GB. BFDV only affects psittacines; therefore, there is no known risk to native British birds. The presence of BFDV in free-living RNPs, however, could pose a disease threat to captive psittacines. Further work is required to determine the distribution and impact of BFDV infection in free-living RNPs in GB. Whether this case represents sporadic disease associated with established endemic infection, or the index case of an emergent disease is currently unknown.

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Psittacine beak and feather disease in a free-living ring-necked parakeet (*Psittacula krameri*) in Great Britain

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Abstract

The ring-necked parakeet (RNP), (*Psittacula krameri*), is an invasive species in Great Britain (GB) which is undergoing rapid population expansion in the wild. Although it has been suggested that RNPs could be a potential source of infectious disease, little research has been done on the pathogens infecting this species in GB. Psittacine beak and feather disease (PBFD), caused by *beak and feather disease virus* (BFDV), is an important infectious disease of psittacines, including captive RNPs, in GB and elsewhere. A wild RNP with marked feather abnormalities observed in an urban garden in London was euthanased and examined post mortem. Plucked contour feathers and pooled liver and spleen were PCR positive for BFDV DNA. Histopathological examination of affected skin demonstrated BFDV-compatible lesions. A feather from another RNP from a different location also was PCR-positive for BFDV. This is the first report of PBFD in a wild free-living bird in GB. BFDV only affects psittacines; therefore, there is no known risk to native British birds. The presence of BFDV in free-living RNPs, however, could pose a disease threat to captive psittacines. Further work is required to determine the distribution and impact of BFDV infection in free-living RNPs in GB. Whether this case represents sporadic disease associated with established endemic infection, or the index case of an emergent disease is currently unknown.

Keywords

Ring-necked parakeet; Psittacine beak and feather disease; Beak and feather disease virus; Invasive species; Great Britain

The ring-necked parakeet (RNP), (*Psittacula krameri*), native to Africa and the Indian subcontinent (Colar, 1997), became established in Great Britain (GB) in the 1960s, where the wild population has rapidly increased over the last decade, particularly in urban areas (Holling et al. 2011). In GB, the majority of RNPs are present in South-East England, with an estimated population of circa 30,000 in the Greater London area (Holling et al., 2011). RNPs are gregarious, forming large communal roosts throughout the year with high rates of social contact. Research into the impact of this invasive species has focused on assessing the significance of interspecific competition with native birds (Newson et al. 2011). Although it has been suggested that RNPs could be a potential source of zoonotic pathogens and diseases of concern to the poultry industry (Fletcher and Askew 2007), little research on the pathogens infecting this species in GB has been reported.

Psittacine beak and feather disease (PBFD), caused by *beak and feather disease virus* (BFDV), genus *Circovirus*, is a well-known disease of captive psittacines in GB and elsewhere. PBFD can affect a range of species, including RNPs (Gerlach 1994), and is considered to be one of the most important infectious diseases of psittacines worldwide (Katoh et al. 2010). The disease most commonly occurs as an insidious, progressive disorder characterised by abnormal feather growth and feather loss, occasionally abnormal beak growth, and often concurrent immunosuppression and secondary infection. Subclinical infection and BFDV shedding (from faeces, crop secretions and feather dander) may occur for several months before feather abnormalities become visible. The virus is thought to be stable outside the host and environmentally persistent (Gerlach 1994; Raidal 2012).

A wild RNP with severe plumage abnormalities was observed in an urban garden in North London, October 2012, where supplementary feeding was practised. The bird was bright and active but struggled to take off from the ground; it was therefore captured and euthanased. RNPs had been regularly observed to visit this site (flocks of 5-20 birds) and the surrounding gardens. This included at least one, perhaps two, other RNPs with feather loss which were seen in the same garden on multiple occasions over a two month period following the euthanasia of the index case.

The euthanased bird was an immature female and was examined post mortem using a standard protocol comprising systematic external and internal examination of body systems, as described by Robinson et al. (2010). Marked feather abnormalities were noted with loss of the majority of contour feathers over the head and

body (Fig 1.). The covert feathers were absent but the primary and secondary flight feathers were present and normal in appearance. The tail feathers were absent. No claw or beak abnormalities were observed. No leg ring or microchip (radiographic examination) was present. The bird was in good body condition with ample fat deposits. The spleen appeared to be enlarged (8.8mm by 8.8mm by 11.4mm) and had a rounded appearance. No other macroscopic abnormalities were observed. Routine microbiological examination of the spleen, liver and small-intestinal contents yielded no significant organisms. Microscopic examination of a wet smear preparation of small-intestinal contents was negative for metazoan and protozoan parasites.

Histopathological examination of the featherless skin from the back revealed focal necrotic and haemorrhagic folliculitis with generalized feather dystrophy and loss. Inclusion bodies consistent with those found in cases of PBFD were observed: intranuclear in the follicular epidermal collar keratinocytes and intracytoplasmic in the feather pulp histiocytes (Fig 2.). Microscopically, the liver contained multiple foci of mild portal, lymphofollicular hepatitis and the spleen was diffusely congested; no abnormalities were detected in the heart, kidney, lung or trachea. Immunohistochemical examinations of the spleen for *Chlamydia* spp. (Buxton et al. 1996) and *Parachlamydia* spp. (Wheelhouse et al. 2012) were negative. DNA extracted from pooled liver and spleen was negative for *Chlamydia* spp. using the 23S arraytube microarray described by Borel et al. (2008).

Plucked contour feathers and pooled liver and spleen were tested commercially for the presence of BFDV DNA using a real-time Taqman PCR assay (Avian Biotech, Florida, USA). A conserved region of the BFDV genome was targeted, with a PCR product of circa 30 bp, using a forward primer sequence of GTGATGTCCGGACGCAAAATG, a reverse primer of AATAGCGACAGGTTATGCAGG and the Taqman Probe: /56-FAM/AAGTCGCGCGAGAGTTCCCNGATAT/3BHQ_1/. The protocol comprised pre-incubation denaturation of extracted DNA at 95^{o}C for 10 min, followed by 10 seconds at 95^{o}C and 60 seconds at 60^{o}C for 40 cycles of duplicate samples. The PCR reaction volume was 10 µL [1 µl sample, 0.5 µl of each primer, 0.5 µl of probe, 3.0 µl of water and 5 µl of the PCR mastermix (New England Biolabs, USA)]. Positive and negative control samples were included with each run. Each set of tissues tested from the affected bird was positive for BFDV DNA.

Two other separate reports of RNPs with feather loss affecting the head and body were received subsequent to the index case. The first was from an urban garden in South London (16.8 kilometres from the North London

incident) in September 2012. The affected bird fed regularly in the garden for six days within a flock of 2-5 parakeets, after which it disappeared. Although no body was retrieved, parakeet feathers consistent with the remains of a predator attack were found within 24 hours of the most recent sighting of the affected bird. One of these feathers was submitted to Avian Biotech (Florida, USA) and was PCR-positive for BFDV. The second report involved a single bird with plumage deficits over the head and body (confirmed by photos) that was seen for a period of 7-10 days in the same garden in North London as the index case (however, one year later, in November 2013). It was observed on the ground, not able to fly and was later predated; however, no samples were available for BFDV testing or post-mortem examination. In addition, a third RNP, found dead due to predation in a third location in South West London (13.0 kilometres from the North London and 14.1 kilometres the South London cases), but with no plumage abnormalities, was examined post mortem. Again, plucked contour feathers submitted to Avian Biotech (Florida, USA) tested PCR-positive for BFDV.

This is the first report of PBFD in free-living RNPs, or any wild bird, in GB. Whilst infections of the genus *Circovirus* have been demonstrated in a range of passerine and non-passerine species, the significance of BFDV to native birds in GB is unknown. BFDV is only known to infect psittacines (Raidal 2012), of which there are no native species in GB; therefore, there is no known risk to native birds. Aviculturalists should be informed of the potential disease risk from free-living RNPs and biosecurity measures to minimise possible disease transmission from wild to captive psittacines should be taken.

Further work is required to determine the distribution and impact of BFDV infection in free-living psittacines in GB. Whether this PBFD case represents sporadic disease associated with established BFDV endemic infection, or the index case~~s~~ of an emergent disease is currently unknown. Although studies by Ha et al. (2007) suggest that wild populations of exotic psittacines may be able to sustain BFDV infection at high prevalence, little is known about the minimum population size required to maintain infection. Nevertheless, it seems plausible that BFDV could persist within the expanding population of RNPs in Greater London that congregate at high density in night roosts of several thousand birds throughout the year (Butler, 2005; H. Peck, *unpublished data*). Horizontal spread is considered likely to be the most important route of transmission of BFDV, however, vertical transmission may also occur from carrier birds (Raidal, 2012). Raidal (2012) has also suggested that the contaminated cavities of hole-nesting psittacines in the wild may play an important role in the epidemiology of BFDV, This could also be the case in GB, where nest-site re-use is frequent by RNPs (Butler et al. 2013).

Full genome sequencing of BFDV from free-living RNPs in GB, as has been performed in other studies (Varsani et al. 2011; Massaro et al. 2012), is required to further investigate the likely source of infection and to provide a baseline for future investigation of the genetic evolution of this pathogen within the British RNP population. Serosurveillance studies would help inform whether BFDV has recently emerged or is established in the wild RNP population in GB (Ha et al., 2007).

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Figure legend

Fig 1 Ring-necked parakeet (*Psittacula krameri*) with extensive feather dystrophy and feather loss over the head and body.

Fig 2 Ring-necked Parakeet (*Psittacula krameri*) feathered skin, Psittacine Beak and Feather Disease (circovirus infection), H&E 400x. The section shows a follicle with epithelial hyperplasia, degeneration of keratinocytes and hyperkeratosis. Macrophages in the pulp show prominent cytoplasmic inclusion bodies (arrows). Basal epithelial cells show marginated chromatin and nuclear inclusion bodies (arrowhead).

Figure
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